PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
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I. INHIBITION OF EGF BINDING

A. Objective: Establish the EGF assay and determine the effects of CSC in the assay.

B. Results: Three replicate experiments were done to examine the effect of 2R1 CSC at 25 ug/ml on the binding of ^{125}I-EGF at doses of 0.1 - 2.0 ng/ml to cultured 3T3 cells (1). The results showed that this concentration of CSC inhibited the binding of EGF by 20% compared to the DMSO solvent control while the positive control (1 ug catechol/ml) inhibited EGF binding by 50%. The DMSO solvent control (2%, v/v) did not inhibit EGF binding. These results are in excellent agreement with results from previous experiments (2).

C. Plans: Evaluate several concentrations of CSC (e.g. 50, 75, 100 ug/ml) using increasing concentrations of ^{125}I-EGF in an effort to obtain representative Scatchard and Lineweaver-Burk plots. This will allow determination of whether the observed inhibition is due to a reduction in the affinity of the binding sites for EGF or a reduction in the number of binding sites per cell or both.

D. References:

1. Stagg, D. L. Notebook 8518, pp. 107-192.
2. Stagg, D. L. Notebook 8518, pp. 67-106.

II. Diacylglycerol (DAG) Assay: Determination of the Maximum Tolerated Dose of 2R1 CSC for Cultured 3T3 Cells

A. Objective: To establish whether treatment of 3T3 cells with high doses of CSC will cause detachment of cells from the culture dishes.

B. Results: Treatments with CSC at concentrations up to 600 ug/ml (2 ml treatment volume) for treatment times of up to 2 hours in 100 mm glass Petri dishes containing 2.8E6 to 4.7E6 cells per dish did not induce any significant cell losses. The cell generation times, however, were longer than expected in this experiment.

C. Plans: Examine factors affecting the growth rate (generation time) of 3T3 cell cultures (see below), then repeat this experiment at higher CSC/cell ratios.

D. References:

Garcia, H. D. Notebook No. 8509, pp. 57-65.

III. DAG Assay: Factors Affecting the Growth Rate of 3T3 Cells in 100 mm Petri Dishes

A. Objective: Determine which culture parameter(s) is rate-limiting for growth of 3T3 cells so as to better define the cultures used in the experiments.

B. Results: The following parameters were examined: glass versus plastic dishes; 10 ml vs 20 ml of culture medium; 10% vs 20% calf serum in the medium; and cell innocula of 5E5, 1E6, 1.5E6 and 2E6 cells per dish. The cultures were all incubated for 3 days, then cell counts were obtained. These counts showed that: 1) these cells grow equally well in glass or plastic dishes; 2) the volume of culture medium is not limiting the rate of cell division under the experimental conditions used; 3) the generation times decrease (i.e., the rate of cell division increases) as the amount of serum per cell is increased.

C. Conclusions: After 3 days of incubation, these cultures appear to have depleted one or more necessary mitogenic factors from the serum, even at the highest serum/cell levels tested. This may prove useful for future experiments if it also means that the baseline level of DAG/cell is lower under these "growth factor depleted" conditions than for cells which are actively dividing.

D. References:

Garcia, H. D. Notebook No. 8509, pp. 66-76.

IV. Acylarachidonyl Glycerol (AAG) in 3T3 Cells: TLC Method Development

A. Objective: Establish conditions for thin layer chromatography of AAG.

B. Results: Two lipid standards, 1-oleoyl-2-acetyl-rac-glycerol (OAG) and 1,2-dioleoyl-rac-glycerol (DOG) were used to determine that 40 ug of sample per spot are needed to clearly visualize spots using iodine development. A solvent mixture of 7:3 toluene:ethyl acetate used to develop the TLCs was found to separate a mixture of these two closely related diacylglycerols.

C. Plans: Use the conditions established above in further experiments involving analysis of unlabelled lipids extracted from 3T3 cell cultures.

D. References:

Nixon, G. Notebook No. 7758, pp. 74-75.

V. Protein Kinase C (PKC) Activity of 3T3 Cells: Electrophoretic Analysis of Protein Extracts from Whole Cells

A. Objective: Determine the baseline electrophoretic pattern of total cell proteins from untreated 3T3 cells.

B. Results: Cells were solubilized, subjected to gel electrophoresis and the gels stained. Good staining patterns for the cell proteins were obtained. Protein analyses were conducted on the cell extract; however, indications were that the high concentrations of SDS in the solubilization media interfered with the protein assay.

C. Plans: Alternate methods of solubilizing the cells and accurately determining protein levels prior to electrophoresis are being investigated.

D. References:

Tickle, M. H. Notebook No. 8515, Pp 105, 117, 129.

VI. PKC Activity in 3T3 Cells: Calibration of Autoradiograms

A. Objective: Optimize the exposure times needed to obtain visible spots on XAR film with various amounts of ^{32}P-ATP.

B. Results: An exposure time of 24 hr at -70°C allowed detection as low as 10 dpm of ^{32}P-ATP.

C. Plans: Additional experiments will be performed to investigate the reproducibility of these conditions.

D. References:

Tickle, M. H. Notebook No. 8515, Pp 124, 144.

VII. Glutathione Depletion Assay (GDA): Reevaluation of Eight Model Cigarettes

A. Objective: To recalculate rate constants (specific activities) using % GSH remaining versus weight of cigarette burned dynamically for the eight model cigarettes.

B. Results: The results from eight model cigarettes were recalculated and evaluated. As the weight of filler burned per puff increased there was an increase in the aldehydes and CO produced. In addition, the more weight burned per puff resulted in less TPM and GP activity. These results suggest that more than just aldehydes are involved in GDA activity.

C. Plans: To recalculate specific activities using % GSH remaining versus weight of cigarette burned dynamically and reevaluate the filter cigarettes designed to remove aldehydes.

D. References:

McCoy, W. R. Notebook No. 8484, pp. 160-163.

VIII. Salmonella/Microsome (S/M) Assay: Effect of Addition of Fructose to Burley CEL on IT CSC Activity

A. Objective: To investigate the effect of total reducing sugars (TRS) in filler on the S/M activity of the CSC.

B. Results: The addition of 6.75% fructose to Bu CEL on BrBW resulted in: (1) 18% decrease in activity with the addition of fructose to CEL (cooked prior to spraying); (2) a 16% decrease in activity with the addition of fructose to CEL (cooked before and after spraying); and (3) no decrease in activity when fructose was added to CEL (no cooking and/or just cooked after spraying). No statistical difference in activity could be demonstrated between control samples without fructose (cooked or uncooked).

C. Plans: To repeat the experiment with glucose and fructose addition levels of 15%.

D. References:

Thompson, L. H. Notebook No. 8516, pp. 97-98.